





a ChIP assay





(a) BALB/c T cells were cultured with anti-CD3e and anti-CD28 mAb for 48 hours by with (dotted bar) or without (solid bar) SAHA and evaluated for T cell expansion with incorporation of [³H] thymidine for the last 18 hours of incubation. Data are the mean \pm SE of quadruplicate cultures. solid vs. dotted bar, P = NS; Data are from 1 of two experiments with similar results.

B6 BM DCs were pretreated with diluent (solid black bar) or 500nM SAHA (dotted bar) for 14-18 hours, were then washed and used as stimulators for BALB/c T cells. Supernatants were collected at 48 hours and used for measuring the levels of (**b**) TGF β and (**c**) IL-10. Solid black bar vs. dotted bar, P = NS for both TGF β and IL-10.

Supplemental Figure 2: Effects on human PBMC derived DCs PBMC derived DCs were obtained and treated with diluent (triangle) or SAHA (circle) and used as stimulators for allogeneic or autologous (square) PBMC from healthy human volunteers as in Materials and methods. (a) Allogeneic proliferation at indicated days of culture is shown. Triangle vs. cirlcle, *, P <0.01 and (b) IFN- γ levels in the supernatants collected at 48 hours from above are shown. Solid bar vs. dotted bar, * *, P <0.05. Data are the mean ± SE of triplicate cultures. PBMC derived DCs from above were with anti-CD11c-FITC and were then gated for PE labeled (c) anti-CD40 and (d) anti-CD 80 on CD11c⁺ cells only. PE staining on CD11c⁺ cells is the dotted line (IgG control), thick dark line represents SAHA treated and the thin line represents control DCs. Results are representative of two replicate experiments.

(a) IL-2 rescues T cell proliferative responses: BALB/c T cells were cultured either with B6 DCs pretreated with diluent (solid bar) or SAHA (dotted bar). IL-2 was added at (5 ng /ml) to some of T cells cultures with SAHA treated DCs (gray bar) and were evaluated for T cell expansion at 72 hours with incorporation of ³H thymidine for the last 24 hours of incubation. Data are the mean \pm SE of quadruplicate cultures. Dotted bar vs. gray bar, * P = 0.0028.

(b) Cell-mediated cytotoxicity assay: BALB/c T cells were stimulated in a bulk MLR with either SAHA treated (open circles) or control DCs (solid circles) for 5 days. They were then harvested and tested for CTL functions by percent lyses of allogeneic B6 Con A blasts as in Materials and methods. Data are the mean \pm SE of quadruplicate cultures. Open circles vs. solid circles, P = NS.

- (a) ChIP assay to assess histone H4 acetylation at the IDO promoter region: BM DCs were treated with diluent or increasing concentrations of SAHA. Chromatin complexes were immunoprecipitated with antibodies to acetylated histones H4 (+) (row 1) or with control rabbit IgG (row 2). PCR primers specific for the IDO promoter were used to amplify the precipitated DNA as in Methods. Row 3 shows the input control in each case.
- (b) B6 mice were given either saline diluent (n = 3) or 5mg /kg of ITF 2357 (n = 3) for 4 successive days. They were then killed and the splenocytes were harvested for total RNA isolation that was reverse-transcribed as in Materials and Methods.